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Constructs						Deletion (nucleotides)	PABV number	M- expression	N- expression
5'UTR- ORF1ab	ORF2	ORF3	ORF4	ORF5	ORF6	ORF7	3'UTR		
							AAA	-	+
							AAA	437	+
							AAA	594	+
							AAA	521	-
							AAA	664	-
							AAA	668	-

^{b)} Identical results were obtained in IPMA using MAbs against GP₃ and GP₄

Fig. 1A

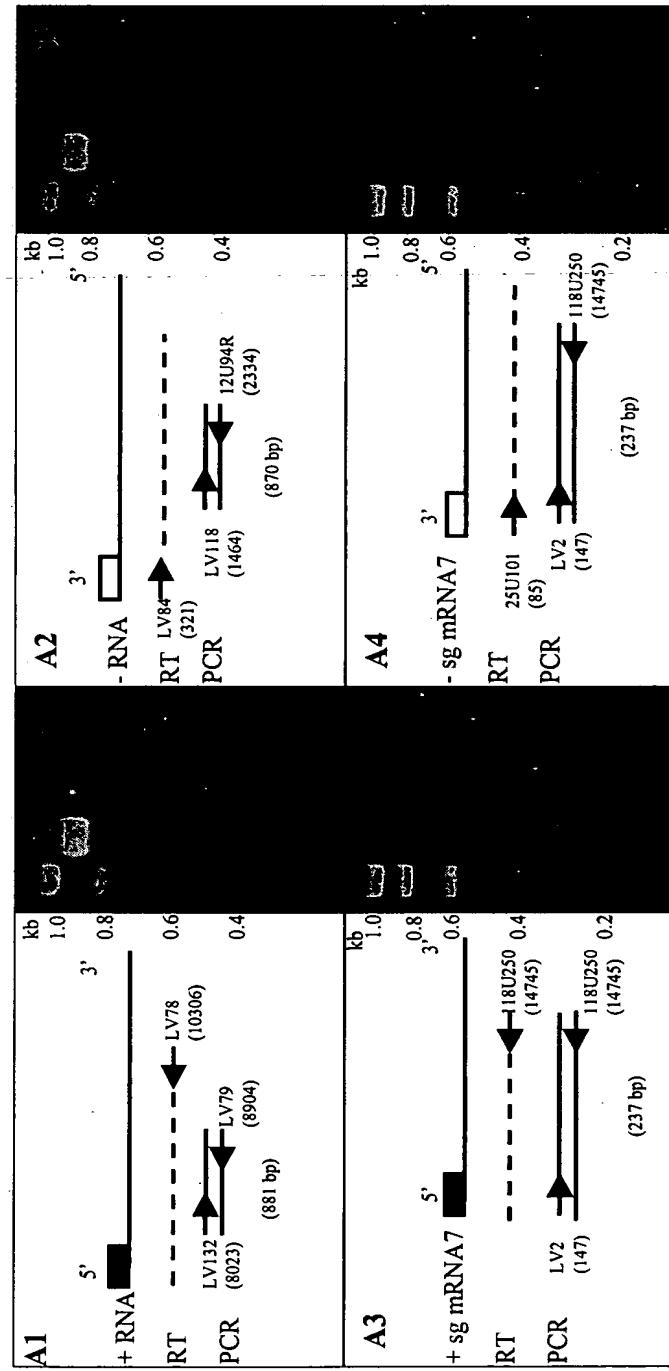
Constructs	Deletion (nucleotides)	PABV number	M- expression
5'UTR- ORF6 <i>Hpa</i> I ATG ORF7 <i>Pac</i> I TAA 3'UTR AAA	-	437	+ ¹⁾
	Δ 14588-14936	605	-
	Δ 14588-14885	604	-
	Δ 14588-14786	603	-
	Δ 14588-14687	602	-
	Δ 14588-14642	624	+
	Δ 14599-14642	625	+
	Δ 14588-14600	626	+ ¹⁾
	Δ 14938-14980	638	+ ¹⁾
	Δ 14887-14980	637	+
	Δ 14788-14980	636	+
	Δ 14686-14980	635	+
	Δ 14643-14686	631	-
	Δ 14643-14676	632	-
	Δ 14643-14664	633	-
	Δ 14643-14652	634	+
	Δ 14653-14686	696	-
	rescue of 696	730	+ ¹⁾

¹⁾ Identical results were obtained in IPMA using MAb 122.17 against N

Fig. 1B

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Fig. 2



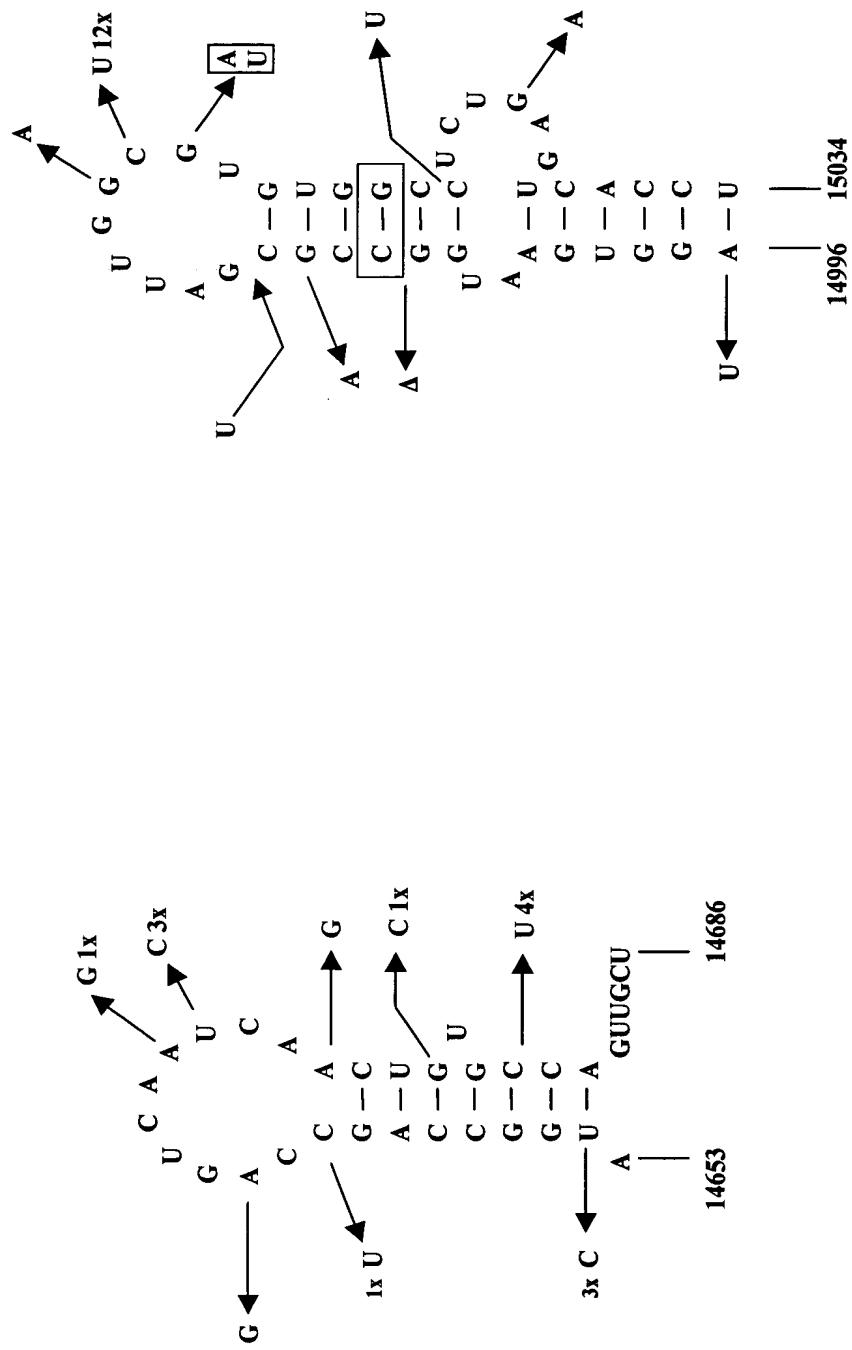
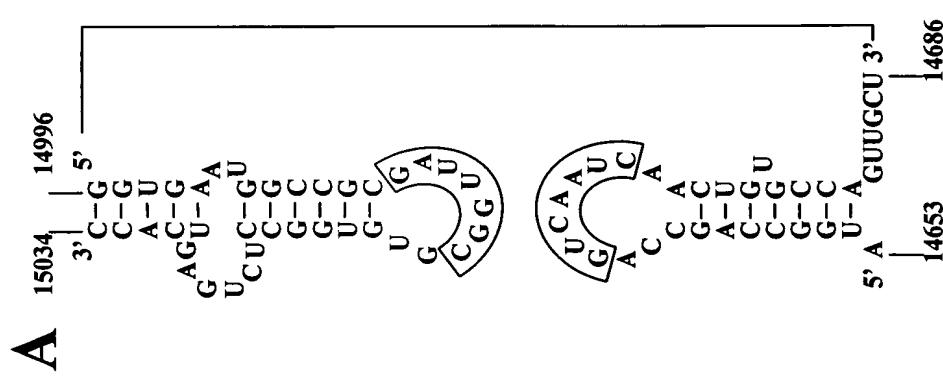
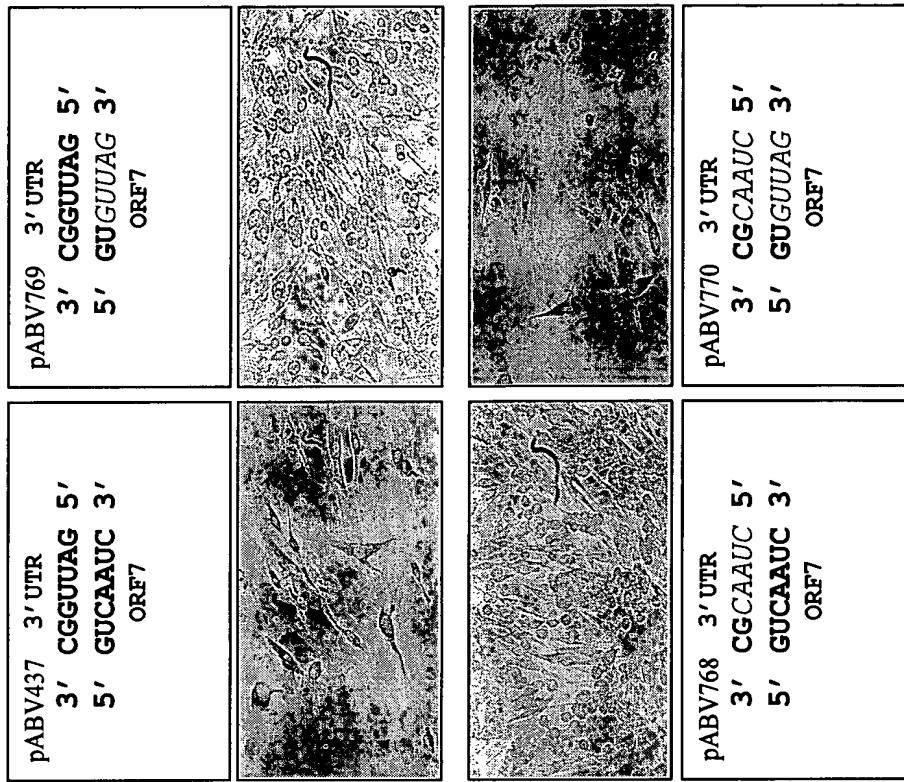


Fig. 3B

Fig. 3A

**B****Fig. 4**

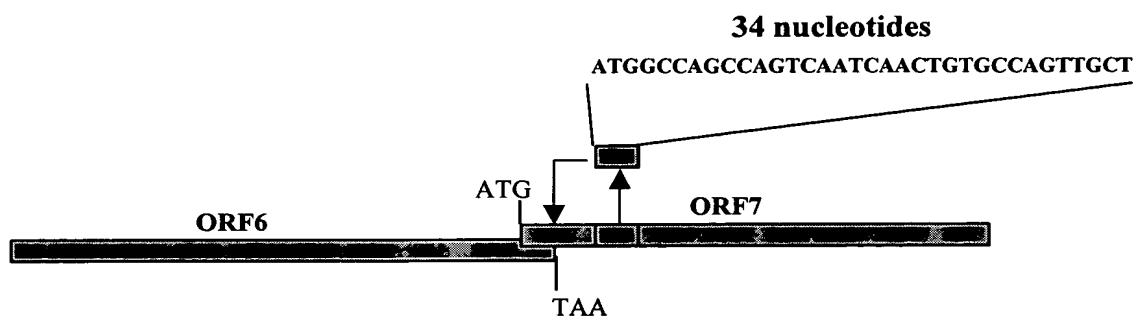


Fig. 5

A

LV	:	MAGKNQSQKKKKSTAPMNGQPVNQLCQLLGAMIKSQRQ	--QPRGGQAKKKPEKPHFPLAEDDIRHH	:	67
VR2332	:	MPNNNGKQQKRK	----KGDGQPVNQLCQLMLGKIIAQQNQSRGKGPGKKNNPEKPHFPLATEDDVRHH	:	66
	*	*	*	*	*

LV	:	LTQTERSLCLOSIIQTAFNQAGTASLSSSGKVSFQVEFMLPVVAHTVRLIRVTSTSASQGAS	:	128	
VR2332	:	FTPSERQLCLSSIQTAFNQAGTCTLSDSGRISYTFEFSLPLTHHTVRLIRVTASPSA	---	:	123
	*	*	*	*	*

B

LV	:	<u>TAAACAGTCA</u>	-----	-----GGTGAATGGCCGCGATGGCG	:	32
VR2332	:	TGGGCTGGCATTCTTGAGGCATCTCAGTGTGTTGAATTGGAAAGAATGTGTGTAATGGCACTGATTGACA	:	70		
	*	*	*	*	*	*

LV	:	TGTGGCCTCTGAGTCACCTTCAATTAGGGGATCACATGGGGTCAACTTAATCAGGCAGGAACCAT	:	102
VR2332	:	TTGTGGCCTCTAAGTCACCTTCAATTAGGGGACCGTGTGGGTGAGATTAAATT-GGCCAGAACCAT	:	139
	*	*	*	*

LV	:	GTGACCGAAATTAAAAAA	:	122
VR2332	:	GCGGCCGAAATTAAAAAA	:	159
	*	*	*	*

Fig. 6

Constructs	Deletion (nucleotides / amino acids)	Plasmid number	M- expression	N- expression	Virus production
ORF7 3'UTR AAA	wild type	437	+	+	+
Δ14975-14980 / Δ 2	639	+	+	+	+
Δ14969-14980 / Δ 4	694	+	+	+	+
Δ14966-14980/ Δ 5	745	+	+	+	+
Δ14963-14980/ Δ 6	746	+	+	+	-
Δ14960-14980/ Δ 7	747	+	+	+	-
Δ14957-14980/ Δ 8	748	+	+	+	-
Δ14954-14980/ Δ 9	695	+	+	+	+
Δ14989-14995	693	+	+	+	-
Δ14989-15020	729	-	-	-	-

Fig. 7

Growth curves of PRRSV deletion mutants

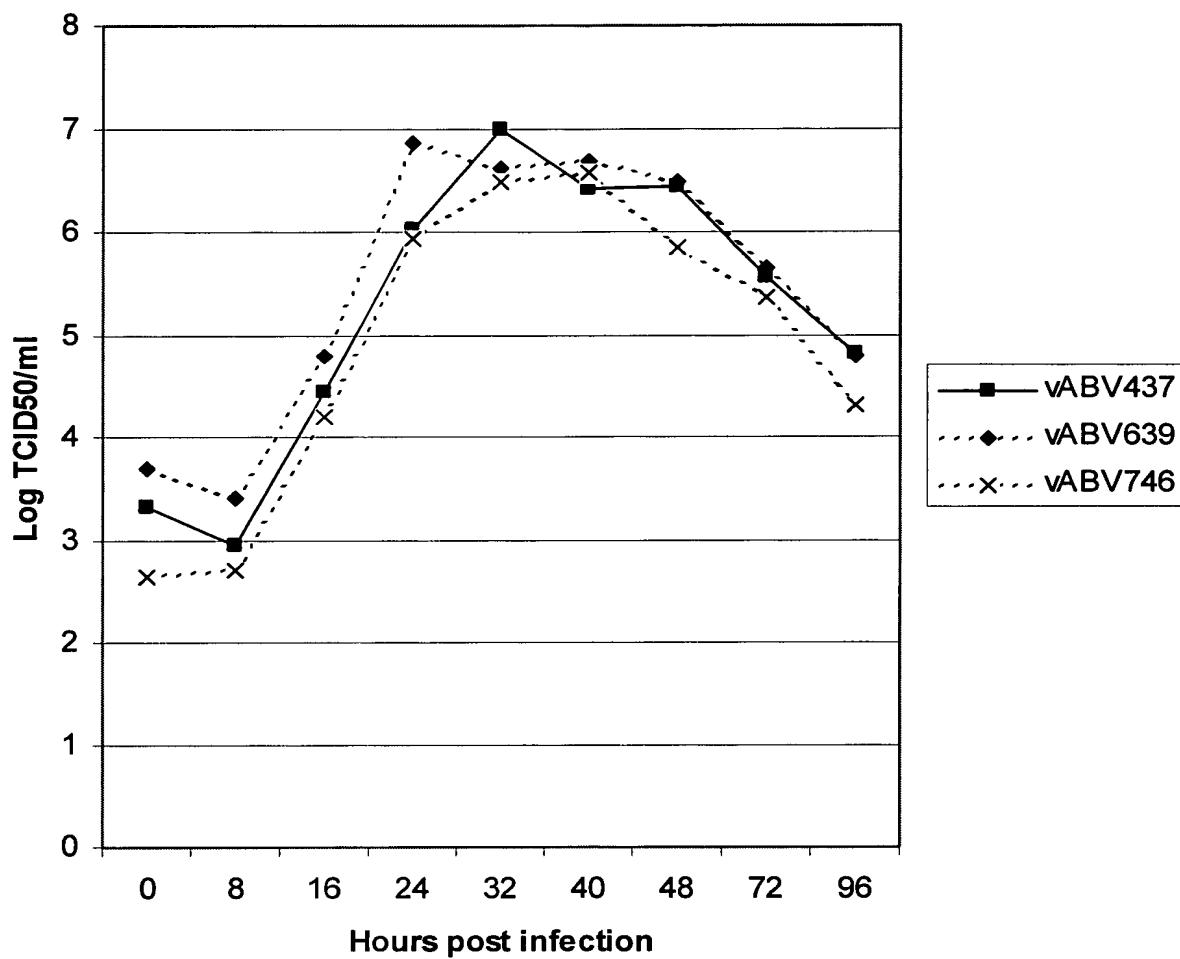


Fig. 8

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Fig. 9

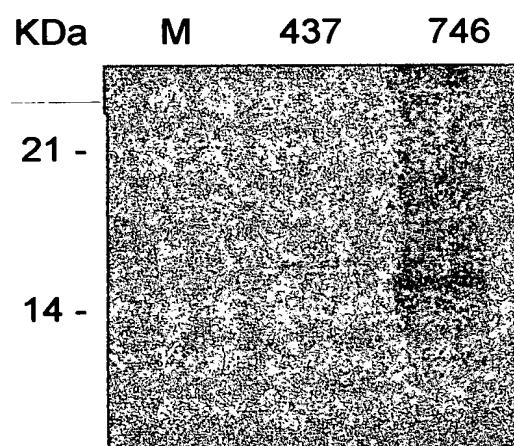


Fig. 10

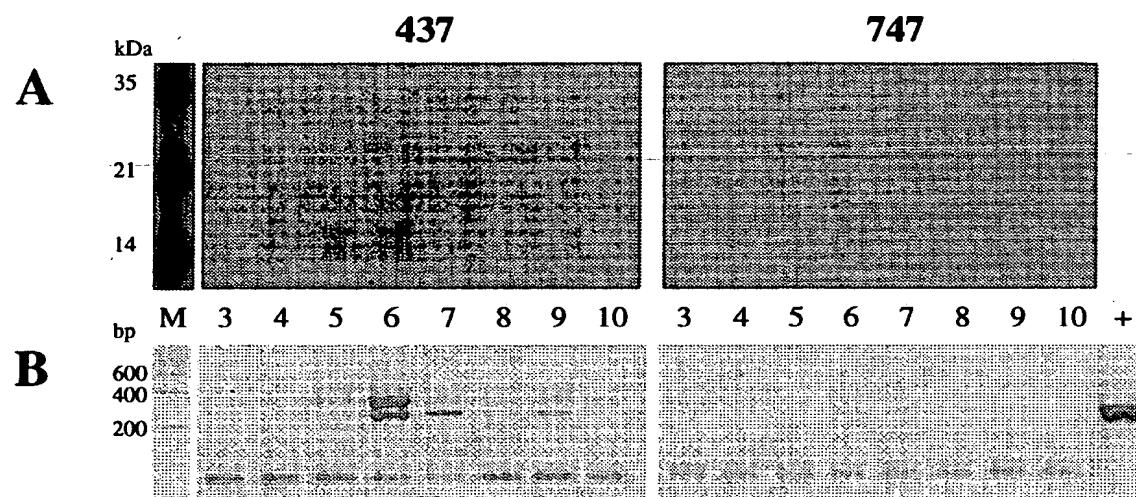


TABLE 1: Sequences of the primers used to introduce deletions by PCR, and primers used to sequence the introduced mutations.

Primer	Sequence of the primer ^a	Orientation	Purpose (pABV)	Location
119R218R	5' ATGACATCCGGCACCA 3'	+	Sequencing	14782
LV20	5' CCTGATTAAGCTTGACCCC 3'	-	Sequencing	15066
LV75	5' TCTAGGAATCTAGACGATCG 3'	-	<i>Xba</i> I-site	15088
LV155	5' <u>ACGTGCGTAA</u> CTCGTCAAGTATGGCCGTAAAAACCAAGGCCAGA 3'	+	<i>Hpa</i> I-site	14582
LV204	5' ACGTGCTTAATTAAACCTTGACTGGGGATGTAGA 3'	-		14974
LV213	5' TGCAAGTTAATTAAAGGTGAATGCCCGCA 3'	+		14996
LV214	5' GACTGTTAATTAAACTGGGGATGTAGA 3'	-		14958
LV215	5' GACTGTTAATTAAAGTCACGCCAATC 3'	-		14942
LV239	5' TGCAAGTTAATTAAAGCTCTGAGTCA 3'	+		15021
LV263	5' GACTGTTAATTAAAGGGATGTAGA 3'	-		14954
LV264	5' GACTGTTAATTAAAGATGTAGAAGTC 3'	-		14951
LV265	5' GACTGTTAATTAAAGTAGAAGTCACG 3'	-		14948
LV266	5' GACTGTTAATTAAAGAAAGTCACGCCA 3'	-		14945

^aThe restriction sites are underlined.

Fig. 12

TABLE 1: Sequences of the primers used to introduce deletions by PCR, primers used to sequence the introduced mutations, and primers used for the strand-specific RT-PCR

Primer	Sequence of the primer ^a	Orien- tation	Purpose (pABV)	Location
118U250	5' CAGCCAGGGAAAATGTTGGC 3'	-	Sequencing / Strand-sp. PCR	14745
120U94R	5' CACCTGACCTGCTCATTTGT 3'	-	Strand-sp. PCR	2334
25U101	5' GTTCTAGCCCCAACAGGTATC 3'	+	Strand-sp. RT	85
LV2	5' AGCGGGAAAGGATCCACCGAGTAT 3'	+	Strand-sp. PCR	147
LV17	5' CCCITGACCGAGCTTCGGC 3'	+	Sequencing	14045
LV20	5' CCTGATTAAGCTGACCC 3'	-	Sequencing	15066
LV75	5' TCTAGGAATCTAGACGATCG	-	PCR <i>Xba</i> 1-site	15088
LV76	5' TCTAGGAATCTAGACGATCG(T40 3'	-	RT	15088
LV78	5' CCCTGGGATGAATCTATGGT 3'	-	Strand-sp. RT	10306
LV79	5' GACAAGATCATCAGGTATACC 3'	-	Strand-sp. PCR	8904
LV84	5' AGAGCTTCAAGGACATGAC 3'	+	Strand-sp. RT	321
LV112	5' CCATTACCTCTACTGTAACTTAACTTGTGACCTCTGA 3'	-	PCR <i>Pac</i> 1-site	14981
LV118	5' TTACCCACCTACTCTCCACCG 3'	-	Strand-sp. PCR	1464
LV132	5' CCTACTGTGCCCTATAGTGTGTC 3'	+	Strand-sp. PCR	8023
LV151	5' ACCAGAGCCAGAAAGAAAAAGTACAGCTGGTGCATAATGAT 3'	+	PCR (631)	14611
LV152	5' ACCAGAGCCAGAAAGAAAAAGTACAGCTGGCAGTTGCTGG 3'	+	PCR (632)	14611
LV153	5' ACCAGAGCCAGAAAGAAAAAGTACAGCTTCAATCAACTGT 3'	+	PCR (633)	14611
LV154	5' ACCAGAGCCAGAAAGAAAAAGTACAGCTATGGCCAGCCAG 3'	+	PCR (634)	14611
LV155	5' ACGTGGGTTAACCTGGTAACTAAGGTGCAATGATAAAAGTCCA 3'	+	<i>Hpa</i> 1-site PCR	14582
LV188	5' ACGTGGGTTAACCTGGTAACTAAGGTGCAATGATAAAAGTCCA 3'	+	PCR (602)	14582
LV189	5' ACGTGGGTTAACCTGGTAACTAAGGTGCAATGATAAAAGTCCA 3'	+	PCR (603)	14582
LV190	5' ACGTGGGTTAACCTGGTAACTAAGGTGCAATGATAAAAGTCCA 3'	+	PCR (604)	14582
LV191	5' ACGTGGGTTAACCTGGTAACTAAGGTGCAATGATAAAAGTCCA 3'	+	PCR (605)	14582
LV195	5' ACGTGGGTTAACCTGGTAACTAAGGTGCAATGATAAAAGTCCA 3'	+	PCR (624)	14582
LV196	5' GGAGTGGGTTAACCTGGTAACTAAGGTGCAATGATAAAAGTCCA 3'	+	PCR (625)	14582
LV197	5' ACGTGGGTTAACCTGGTAACTAAGGTGCAATGATAAAAGTCCA 3'	+	PCR (626)	14582
LV198	5' GCTCGTGTCTAGCCCTTACATACATACAC 3'	+	<i>Nhe</i> 1-site PCR	14140
LV200	5' ACGTGCTTAATTAACCCAGGAACACTGGCAGCTG 3'	-	PCR (635)	14981
LV201	5' ACGTGCTTAATTAATGTCATCTCTAGCCAG 3'	-	PCR (636)	14981
LV202	5' ACGTGCTTAATTAACCCGCTGGATCAAAGGGAGGC 3'	-	PCR (637)	14981
LV203	5' ACGTGCTTAATTAACCCGACTGTAGAGCAACGG 3'	-	PCR (638)	14981
LV204	5' ACGTGCTTAATTAACCCGACTGTAGAGCAACGG 3'	-	PCR (639)	14981
LV216	5' ACCAGAGCCAGAAAGAAAAAGTACAGCTCCGATGGGGAG GGTGCAATGAT 3'	+	PCR (696)	14611
LV268	5' ACCAGAGCCAGAAAGAAAAAGTACAGCTCCGATGGGGAG 3'	+	PCR (769)	14611
LV269	5' CTCGATGGGAATGCCAGGTGTTAGAACCTGTCAGT 3'	+	PCR (769)	14641
LV270	5' TGCAAGTTAATTAACAGTCAGGTGAATGGCGCTTAACCGCTGTGGCCTC 3' +	+	PCR (768)	14981

^aThe restriction sites are underlined.